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THE HISTOGENESIS AND EVOLUTION OF INTEGUMENTS IN ONAGRACEAE[1]

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ABSTRACT

The mode of initiation and subsequent growth of the inner (i.i.) and outer (o.i.) integuments were examined and compared in 40 species, representing all seven tribes and all 17 genera of Onagraceae. The i.i. is of dermal origin and two-layered throughout the family. Four different types of developmental mode of the o.i. are characterized: 1) o.i. of subdermal origin—*Lopezia* (Lopezieae), *Fuchsia* (Fuchsieae), and *Circaea* (Circaeeae); 2) o.i. of both dermal and subdermal origin, with derivatives of the subdermal cells dividing more actively than those of the dermal cells—*Oenothera* and *Stenosiphon* (Onagreae); 3) o.i. of both dermal and subdermal origin, with derivatives of the subdermal cells dividing less actively than those of the dermal cells—*Hauya* (Hauyeae) and *Calylophus* and *Gaura* (Onagreae); 4) o.i. of dermal origin—*Ludwigia* (Jussiaeeae); *Boisduvalia* and *Epilobium* (Epilobieae); and *Camissonia, Clarkia, Gayophytum, Gongylocarpus, Heterogaura,* and *Xylonagra* (Onagreae). Except for the unexpected close similarity of *Hauya* (Hauyeae) to *Calylophus* and *Gaura* (Onagreae), among which is shared an apparently derived state of this character rather suggesting a common ancestry, the patterns of relationships suggested on the basis of the developmental mode of the o.i. closely accord with those derived from other lines of evidence. The results from Onagraceae confirm that the histogenetic characters of integuments are valuable indicators of relationship at a generic level.

The family Onagraceae, composed of seven tribes, 17 genera, and about 674 species (Raven, 1979), is one of the most intensively investigated families of angiosperms. In recent years, in addition to systematic studies (e.g., Raven, 1963, 1964, 1969; Plitmann et al., 1973), there have also been studies on chromosome number and morphology (e.g., Kurabayashi et al., 1962; Raven & Gregory, 1972; Raven & Tai, 1979), wood anatomy (Carlquist, 1975, 1977, 1982), pollen morphology (e.g., Skvarla et al., 1975, 1978), floral anatomy (Eyde & Morgan, 1973; Eyde, 1977, 1978, 1981, 1982), and leaf anatomy (Keating, 1982). As a result of these detailed studies, our knowledge of phylogenetic relationships within the family at a generic level has become quite detailed.

The major outlines of the pattern of relationships that have been revealed are as follows. *Ludwigia* represents a line distinct from all other genera of the family (Eyde, 1977, 1978, 1981; Raven & Tai, 1979). It shares with two of the more primitive genera in the other phylogenetic branch of the family, namely *Fuchsia* (Fuchsieae) and *Hauya* (Hauyeae), the generalized characteristic of lacking interxylary phloem (Carlquist, 1975, 1977). In addition to *Fuchsia* and *Hauya, Lopezia* (Lopezieae) and *Circaea* (Circaeeae), in

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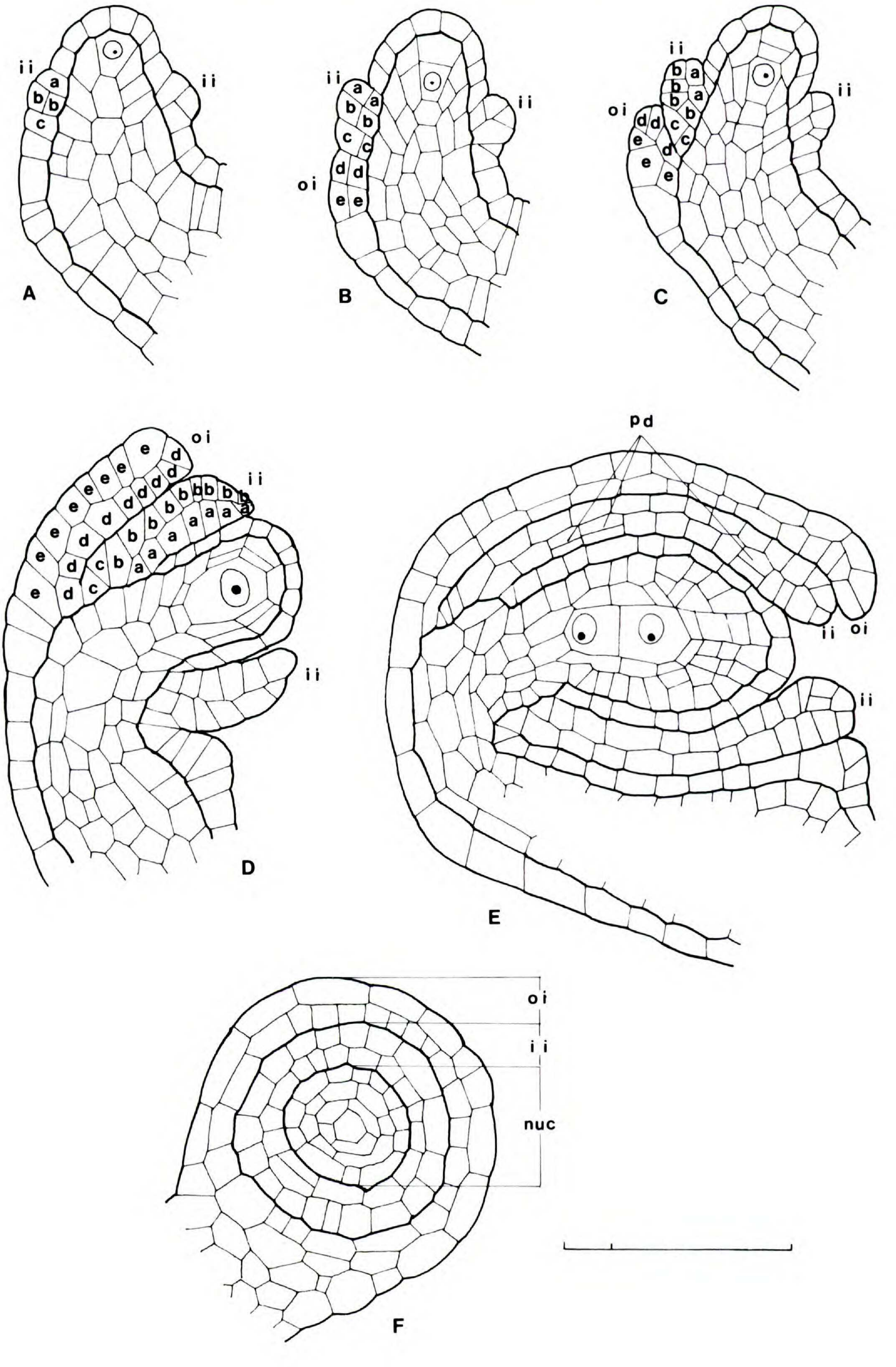
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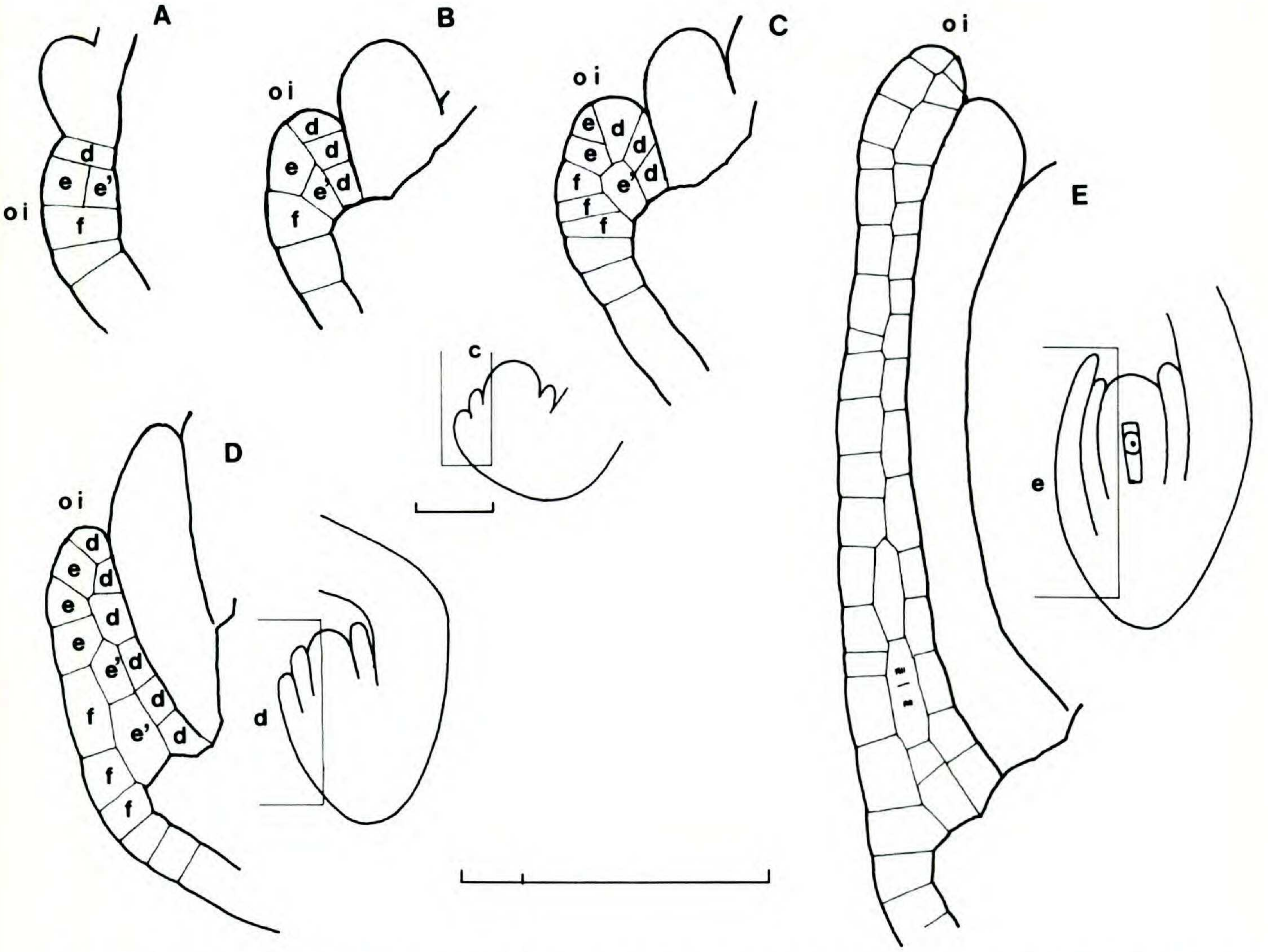

FIGURE 2. *Ludwigia peploides.*—A–E. Median longisections of the o.i. in progressively successive stages of development, showing the development of a distinctive three-layered part of the o.i. d, e, f, dermal initials of the o.i.; e′, daughter cell of the dermal initial e, and functioning as an initial for the third (middle) layer of the o.i. Bracket scales = 100 μm.

which at least the most primitive species have a basic chromosome number of $n = 11$, present an array of relatively primitive characteristics, so that *Circaea, Fuchsia, Hauya,* and *Lopezia* may be regarded as distantly related, generalized lines within this major branch of the family—the branch that does *not* include *Ludwigia.* Epilobieae, consisting of *Boisduvalia* and *Epilobium* only, is advanced in many of its characteristics but occupies a rather isolated position within the family (Raven, 1976). Within the group of ten genera recognized as Onagreae (Raven, 1964, 1969), *Clarkia* and *Heterogaura* constitute one distinctive group; *Oenothera* and *Stenosiphon* another; *Calylophus* and *Gaura* a third; and *Camissonia, Gongylocarpus, Gayophytum,* and *Xylonagra* a fourth (Tobe & Raven, 1985). *Hauya* (Hauyeae) has been separated as a distinct tribe by virtue of its generalized characteristics including the presence of stipules, generalized leaf anatomy distinct from that of all Onagreae (Keating, 1982), and lack of interxylary phloem. On the other hand, its possession of obviously divided sporogenous tissue generally similar to

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FIGURE 1. *Ludwigia arcuata.*—A–D. Median longisections of ovule primordia in progressively successive stages of development, showing that both the inner (i.i.) and outer (o.i.) integuments are of dermal origin. a, b, c, dermal initials of the i.i.; d, e, dermal initials of the o.i.—E. Median longisection of a growing ovule, in which periclinal cell divisions (pd) take place in the inner layer of the i.i.—F. Cross section of a growing ovule. nuc, nucellus. Bracket scale = 100 μm.

TABLE 1. A comparison of histogenetic characteristics of integuments in Onagraceae. Degree of participation of derivatives of subdermal initials in formation of the outer integument (o.i.) defined as follows: (+++) up to the tip of o.i. (see Fig. 10A); (++) more than half as much as, but less than, the whole length of o.i. (see Fig. 10B); (+) up to only the basal part of o.i. (see Fig. 10C); (−) no derivatives of subdermal initials contribute to the formation of o.i. (see Fig. 10D).

Taxon	Locality and Collection Information	Number of Cell Layers in Integument		Origin of Outer Integument	Degree of Participation of Derivatives of Subdermal Initials in Formation of Outer Integument
		Inner	Outer		
Tribe Jussiaeeae					
Ludwigia arcuata Walt.	U.S.A. Florida: Hillsborough Co., *Peng 4320* (MO).	2	2	dermal	−
L. bonariensis (Mich.) Hara	Argentina. Buenos Aires: La Plata, *Ramamoorthy 1005* (MO).	2	2	dermal	−
L. peploides (H.B.K.) Raven	U.S.A. Missouri: St. Louis Co., *Tobe,* 1981 (MO).	2	2–3	dermal	−
L. lanceolata Ell.	U.S.A. Georgia: McIntosh Co., *Peng 4139* (MO).	2	2	dermal	−
L. linearis Walt.	U.S.A. Louisiana: St. Tammany Parish, *Dille 420* (MO).	2	2	dermal	−
L. leptocarpa (Nutt.) Hara	Brazil. Bentos: Santa Catarina, *Ramamoorthy 1134* (MO).	2	2	dermal	−
L. virgata Michx.	U.S.A. Alabama: Mobile Co., *Tobe,* 1981 (MO).	2	2	dermal	−
L. peruviana (L.) Hara	Australia. Mascot (7 km S of Sydney), *Briggs 7143* (NSW).	2	2	dermal	−
L. latifolia (Benth.) Hara	Costa Rica. Puntarenas, *Raven 21575* (MO).	2	2	dermal	−
Tribe Lopezieae					
Lopezia langmaniae Miranda	Mexico. Chiapas, *Breedlove 32300* (CAS).	2	3–4	subdermal	+++
L. racemosa Cav. subsp. *racemosa*	Mexico. Chiapas, *Breedlove 7030* (CAS).	2	3–4	subdermal	+++
L. semeiandra Plitmann, Raven & Breedlove	Mexico. Sinaloa, *Breedlove 8044* (CAS).	2	3–4	subdermal	+++
Tribe Fuchsieae					
Fuchsia radicans Miers.	Brazil. São Paulo: Campas de Jordão, *Ramamoorthy 676* (MO).	2	3–6	subdermal	+++
F. jimenezii Breedlove, Berry & Raven	Costa Rica. Monteverde Preserve, *Haber, Baker & Baker 434* (MO).	2	3–4	subdermal	+++

TABLE 1. Continued.

Taxon	Locality and Collection Information	Number of Cell Layers in Integument		Origin of Outer Integument	Degree of Participation of Derivatives of Subdermal Initials in Formation of Outer Integument
		Inner	Outer		
F. microphylla H.B.K. subsp. *quercetorum* Breedlove	Cultivated, Univ. Calif. Bot. Gard. (Berkeley), *Raven*, 1969 (MO).	2	3–6	subdermal	+++
Tribe Circaeeae					
Circaea cordata Royle	Cultivated, Missouri Bot. Gard. #762431, original plants collected by Raven in 1975 from Vladivostok, USSR (MO).	2	3–5	subdermal	+++
C. alpina L. subsp. *pacifica* (Asch. & Mag.) Raven	Cultivated, Univ. British Columbia Bot. Gard. (UBC).	2	3–5	subdermal	+++
Tribe Hauyeae					
Hauya elegans DC. subsp. *elegans*	Mexico. Chiapas, *Breedlove 42631* (MO).	2	2–5	dermal and subdermal	+
H. heydeana Donn. Smith	Mexico. Chiapas: Ocotal Grande, *Breedlove 15669* (MO).	2	2–5	dermal and subdermal	+
Tribe Onagreae					
Gongylocarpus fruticulosus (Benth.) Raven & Breedlove	Mexico. Baja California: Magdalena Is., *Verity 037* (MO).	2	2	dermal	–
G. rubricaulis Schlecht. & Cham.	Mexico. Chiapas, *Breedlove 41880* (CAS).	2	2	dermal	–
Gayophytum humile Juss.	U.S.A. Oregon: Jefferson Co., *Chambers 4834* (OSC).	2	2	dermal	–
G. heterozygum Lewis & Szwey.	U.S.A. Oregon: Jefferson Co., *Chambers 4835* (OSC).	2	2	dermal	–
Xylonagra arborea (Kellogg) Donn. Smith & Rose	Mexico. Baja California, *Verity, Nakai & Angel*, 1979, no voucher.	2	2	dermal	–
Camissonia ovata (Nutt. ex Torr. & A. Gray) Raven	U.S.A. California: Marin Co., *Raven & Raven 26148* (MO).	2	2	dermal	–
C. californica (Nutt. ex Torr. & A. Gray) Raven	Cultivated, UCLA Bot. Gard., *Verity*, no voucher.	2	2	dermal	–

TABLE 1. Continued.

Taxon	Locality and Collection Information	Number of Cell Layers in Integument		Origin of Outer Integument	Degree of Participation of Derivatives of Subdermal Initials in Formation of Outer Integument
		Inner	Outer		
Calylophus lavandulifolius (Torr. & A. Gray) Raven	U.S.A. Nevada: Lincoln Co., *Tiehm & Williams 6572* (MO).	2	2–3	dermal and subdermal	+
C. serrulatus (Nutt.) Raven	U.S.A. Kansas: Sherman Co., *Brooks 15533* (KANU).	2	2–3	dermal and subdermal	+
Gaura coccinea Pursh	U.S.A. Texas: Pecos Co., *Powell 3589* (MO).	2	2–4	dermal and subdermal	+
G. longiflora Spach	U.S.A. Missouri: Jefferson Co., *Wagner, Mill & Tobe 4522* (MO).	2	2–3	dermal and subdermal	+
Oenothera flava (A. Nels.) Garrett subsp. *flava*	Mexico. Durango, *Wagner & Solomon 4321* (MO).	2	2–5	dermal and subdermal	++
O. villosa Thunb. subsp. *villosa*	U.S.A. Missouri: St. Louis Co., *Wagner, Chin & Tobe 4519* (MO).	2	2–5	dermal and subdermal	++
Stenosiphon linifolius (Nutt.) Heynh.	U.S.A. Oklahoma: McClain Co., *Sullivan 1038* (OKL).	2	2–4	dermal and subdermal	++
Clarkia delicata (Abrams) Nels. & Macbr.	Mexico. Baja California, *Moran 27640* (MO).	2	2	dermal	–
C. tenella (Cav.) Lewis subsp. *tenella*	Chile. Malleco: Curacao, *Marticorena & Ovezada 1669* (MO).	2	2	dermal	–
Heterogaura heterandra (Torr.) Coville	U.S.A. California: Tuolumne Co., *Gottlieb*, 1977 (MO).	2	2	dermal	–
Tribe Epilobieae					
Boisduvalia subulata (Ruiz. & Pavón) Raimann	Chile. Ñuble, *Cheese & Watson 4405* (K).	2	2	dermal	–
B. glabella (Nutt.) Walp.	U.S.A. California: Yolo Co., *Crampton 9212* (MO).	2	2	dermal	–
Epilobium ciliatum Raf. subsp. *watsonii* (Barbey) Hoch & Raven	U.S.A. California: Marin Co., *Sharp*, 1967 (MO).	2	2	dermal	–
E. canum (Greene) Raven subsp. *canum*	Cultivated, Univ. Calif. Bot. Gard. (Berkeley) UCBG 58.996 (UC).	2	2	dermal	–

that found in *Calylophus, Clarkia, Gaura,* and *Heterogaura,* has been used as evidence for linking it with these groups (Raven, 1964, 1969, 1979), as have its vespertine flowers, long floral tubes, white petals, and strong floral odor, this syndrome of characteristics closely resembling that found in white-flowered species of *Oenothera.*

The histogenesis of integuments which is the subject of this paper is used here as another approach to clarify the relationships between the genera of Onagraceae. Although such characteristics have traditionally been included within the scope of investigations of embryologists, they have largely been confined to considerations of the number and thickness of the integuments, the development of integuments into seed coats, and the participation of integuments in formation of the micropyle. In contrast, little attention has been devoted to the developmental mode of the outer and inner integuments (see reviews in Bouman, 1971, 1974, 1984). Twenty-five years ago, the studies of Roth (1957) on *Capsella* began to interest embryologists again in the developmental mode of integuments, a field that had been largely neglected since the studies of Warming (1878) over a century ago. The subsequent works of Bouman and his collaborators on *Juglans* and *Pterocarya* (Boesewinkel & Bouman, 1967), on Polycarpicae (Bouman, 1971, 1977, 1978; Boer & Bouman, 1972, 1974), and on Rutaceae (Boesewinkel, 1977, 1978; Boesewinkel & Bouman, 1978) have increasingly called our attention to the histogenetic characters of integuments as a probable indicator of affinity among taxa. Bouman (1971) emphasized the importance of observations of the histogenesis of integuments in their evaluation, citing the fact that seemingly identical structure of integuments often developed as a result of different histogenetic processes. He also warned against the taxonomic value of data based on the structure of the mature seed coat for similar reasons. For example, *Drimys winteri* Forst. (Winteraceae) has an outer integument of dermal origin (Boer & Bouman, 1974), whereas *Magnolia stellata* (Sieb. & Zucc.) Maxim., *M. virginiana* L., and *Liriodendron tulipifera* L. (Magnoliaceae) have an outer integument of subdermal origin (Boer & Bouman, 1972; Bouman, 1977). This difference in the histogenetic origin of the outer integument has been used as additional evidence for separating Winteraceae from Magnoliaceae, with which they were combined earlier (Boer & Bouman, 1974).

We have made observations of the histogenesis of the integuments as part of a study of the embryology of Onagraceae. In this paper we shall deal only with the mode of initiation and subsequent growth of the integuments, not considering the more diversified morphology of the seed coat or testa. We shall utilize the mode of initiation and subsequent growth of integuments solely as an indicator of affinity between taxa and shall attempt neither morphological interpretations of these structures nor detailed studies of other aspects of embryology in these plants.

Only one earlier paper deals with the histogenesis of integuments of Onagraceae (Geerts, 1908). Geerts provides excellent illustrations of the young ovules of *Oenothera glazioviana* Mich. (= "*O. lamarckiana*"), stating "Das innere Integument entwickelt sich nur aus Dermatogenzellen, zur Bildung des äusseren Integumentes finden im Periblem Teilungen statt." Several other papers dealing with the embryology of Onagraceae (e.g., O'Neal, 1923, *Oenothera biennis* L. = "*O. rubrinervis*"; Johansen, 1934, *Circaea alpina* subsp. *pacifica* = "*C. pacifica*"; Täckholm, 1914, *Lopezia racemosa* subsp. *racemosa* = "*L. coronata*") have illustrations of sections of young ovules including views of developing integuments; all of these papers, however, lack an explanation of the histogenesis of the structures illustrated and are not detailed enough to be used in connection with this present comparative study.

MATERIALS AND METHODS

Forty species representing all seven tribes and all 17 genera of Onagraceae were included in our study. They are listed in Table 1, along with the results obtained from each. All the samples were fixed in FAA (90 parts 50 or 70% ethanol, 5 parts acetic acid and 5 parts formalin) and then dehydrated through tertiary-butyl alcohol series and embedded in paraplast. Serial microtome sections of ovaries 5–7 μm thick were stained with Heidenhain's hematoxylin, safranin, and fast green FCF.

In all Onagraceae, the ovule has two integuments, inner (i.i.) and outer (o.i.); it becomes anatropous or hemitropous (*Lopezia*) at maturity. The terminology, abbreviation of terms, and the manner of drawing microtome sections basically follow Bouman (1974). In every diagram illustrating the histogenesis of integuments, Figures 1, 2, 4–8 for example, bold lines are used

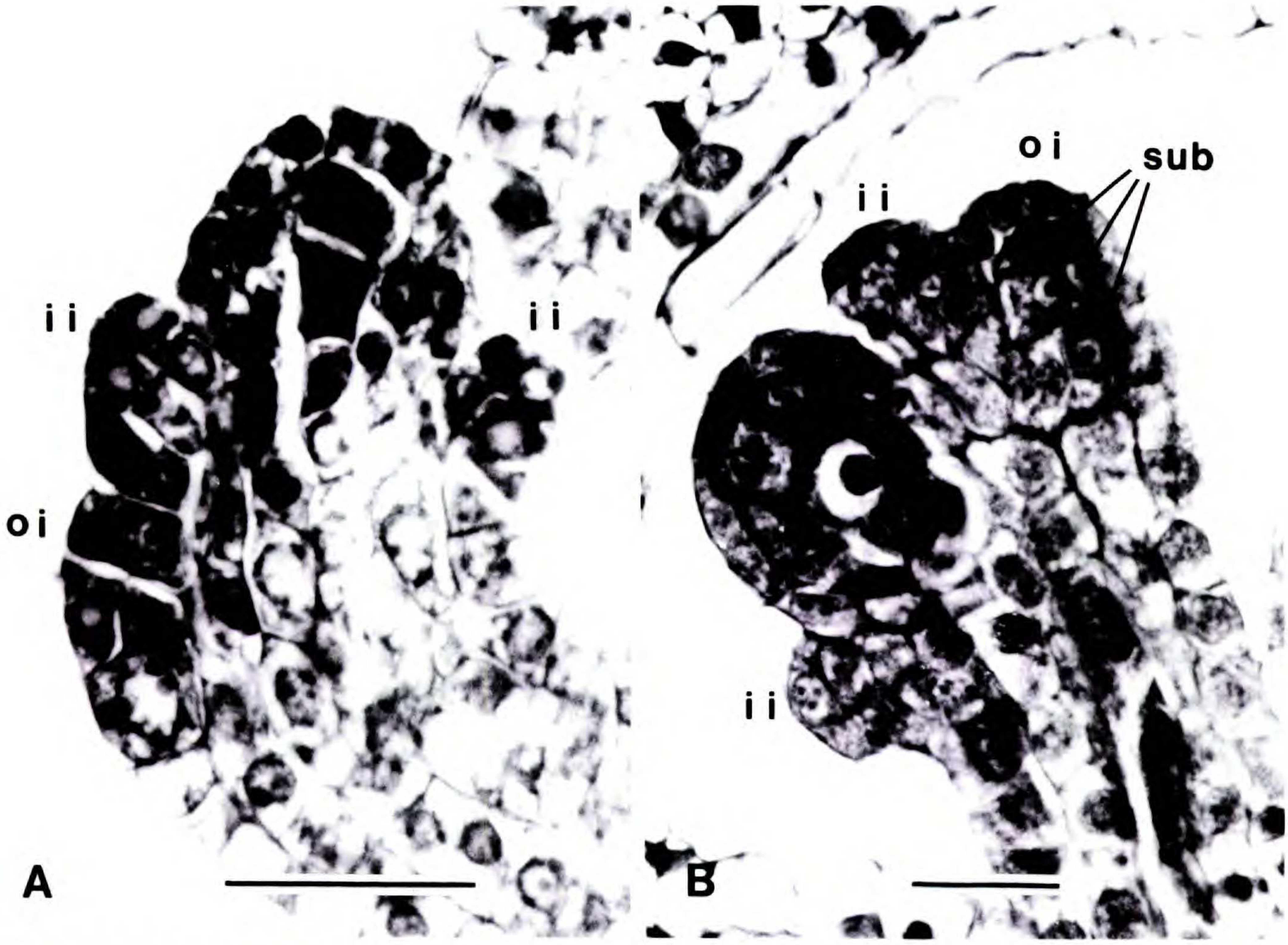

FIGURE 3. Median longisections of the ovule primordia, showing two contrasting developmental modes of the o.i.—A. *Ludwigia arcuata.* Note that the o.i. is initiated dermally.—B. *Lopezia racemosa.* Note that the o.i. is initiated subdermally. Scales = 20 μm.

to represent the boundary between dermal and subdermal tissues.

OBSERVATIONS

TRIBE JUSSIAEEAE

Ludwigia arcuata, L. bonariensis, L. latifolia, L. leptocarpa, L. linearis, L. peploides, L. peruviana, and *L. virgata*—In every species the initiation of the i.i. commences by periclinal divisions of dermal cells located at the flank of an ovule primordium (Fig. 1A, see cells b). In median longisections of the ovule primordium, three dermal initial cells (a, b, c in Fig. 1B; see also Fig. 3A), or occasionally only two, a and b, were recognized. The first periclinal divisions of these three initials form a swelling of the i.i., first discernible externally. The subsequent growth of the i.i. primordium takes place chiefly by divisions of daughter cells of two, a and b, initials (Fig. 1C, D), so that the i.i. grows as a two-layered thin organ. In a developed ovule the i.i. usually becomes three-layered because of periclinal divisions of cells of the inner layer of the i.i. (Fig. 1E, F). The tip of the i.i. ultimately becomes multilayered, forming an endostome similar to that known in many other angiosperms.

Thus, since the initiation and the subsequent growth of the i.i. takes place by divisions of dermal initials and their derivatives, the i.i. of *Lud-*

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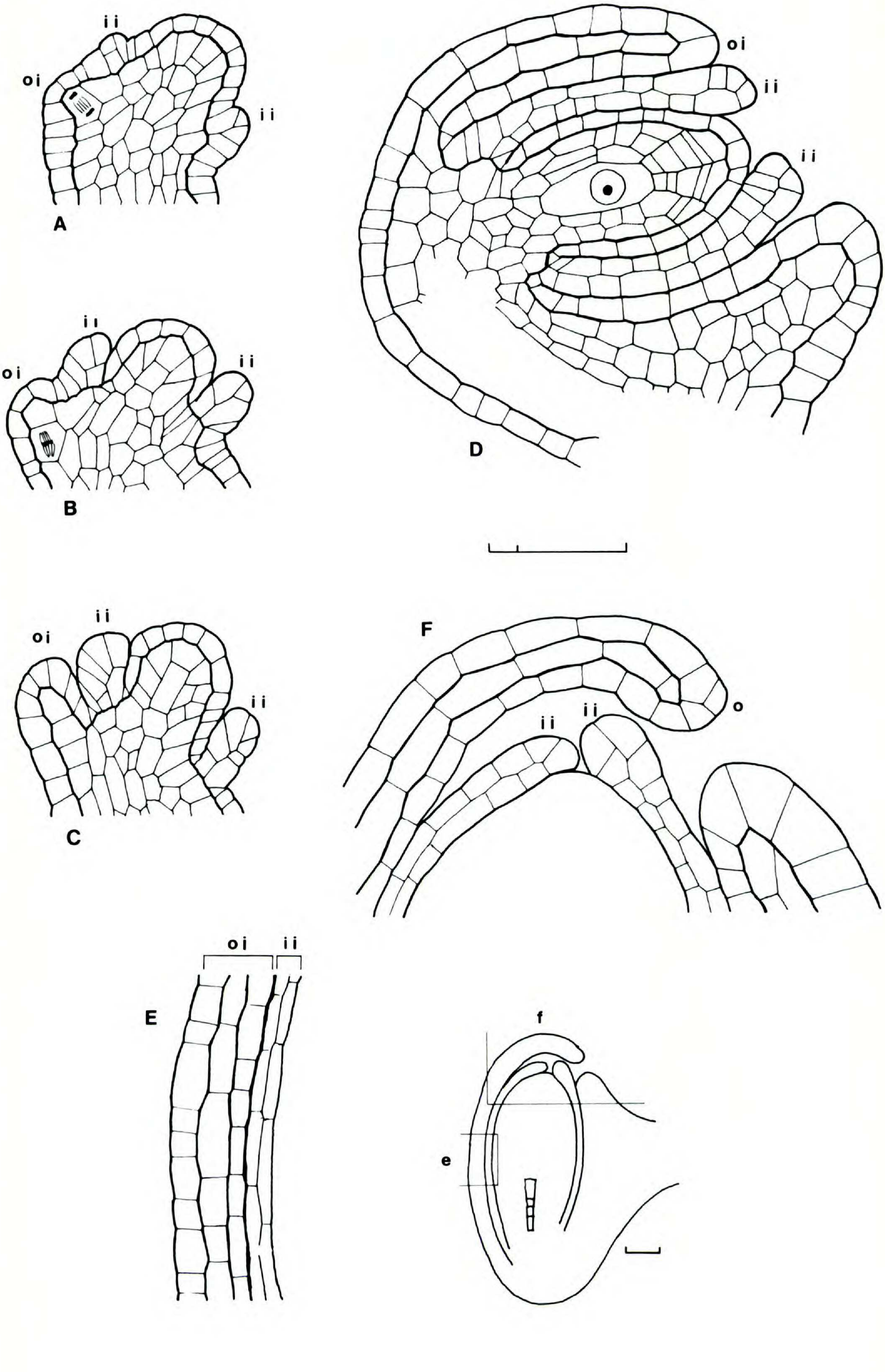
FIGURE 4. *Lopezia semeiandra.*—A–C. Median longisections of ovule primordia in progressively successive stages of development, showing that the i.i. is of dermal origin but the o.i. of subdermal origin.—D. Median longisections of a growing ovule.—E. Longisection of a lateral part of a developed ovule.—F. Longisection of a micropylar part of a developed ovule. Note that the derivatives of the subdermal initials reach up to the tip of the o.i. Bracket scales = 100 μm.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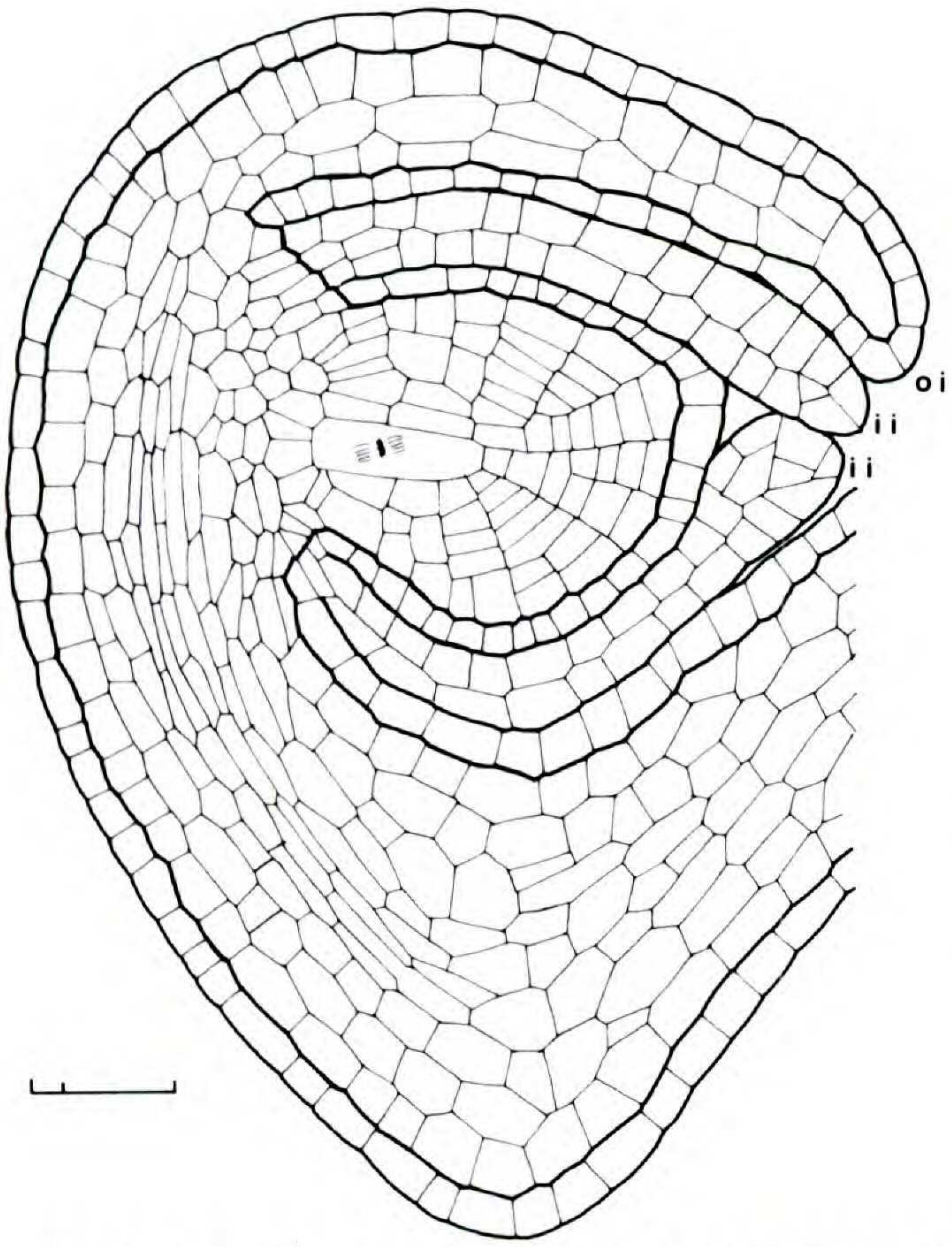


FIGURE 5. *Fuchsia radicans.* Note that the o.i. is of subdermal origin and the derivatives of the subdermal initials reach up to the tip of the o.i. Bracket scale = 100 μm.

wigia is of dermal origin, a term used consistently by Bouman and his collaborators to express this pattern of development.

The o.i. is also of dermal origin in all species examined. At the initiation stage, two dermal initials (d, e in Fig. 1B; see also Fig. 3A) undergo periclinal divisions. Subsequent growth takes place by repeated divisions of derivatives of the dermal initials (Fig. 1C, D). The mature o.i. is two-layered in all the species except *L. peploides* (Fig. 1E, F), and only its tip becomes multilayered to form an exostome. The o.i. hardly develops at the funicular side, and, if it does, it is made up on this side of derivatives of dermal initials only (Fig. 1E).

In the sole exceptional species, *L. peploides,* a developed o.i. is two-layered in the upper part and three-layered in the lower part (Fig. 2E). This three-layered part of the o.i. is formed in a different mode from that of the i.i. The first periclinal division of the dermal initial e gives rise to two daughter cells, the lower cell e′ functioning as a distinct initial of the third (middle) layer in the subsequent growth (Fig. 2A, B). Consequently, the upper part of the o.i. grows by divisions of the upper daughter cell e as well as of another initial d, and the lower part by divisions of derivatives of an additional initial f as well as of e′ and d (Fig. 2C, D).

TRIBE LOPEZIEAE

Lopezia langmaniae, L. racemosa and *L. semeiandra*—The i.i. is of dermal origin and two-layered in all species examined (Fig. 4A–F).

The mode of initiation and the subsequent growth of the o.i. is consistent in all species examined, differing completely from the pattern seen in *Ludwigia.* On the antiraphial and lateral sides, the initiation of the o.i. commences by periclinal divisions of subdermal cells, and not by those of dermal cells (Fig. 4A). Repeated periclinal divisions of subdermal cells give rise to a primordium of the o.i. discernible externally as a swelling (Fig. 4B, C; see also Fig. 3B). Subsequent growth proceeds by divisions of both subdermal and dermal cells (Fig. 4D). Thus, even if dermal cells will have divided periclinally to make an exostome at the tip of the o.i., derivatives of the subdermal initials reach the tip on all antiraphial and lateral sides (Fig. 4F). The mature o.i. is usually three-layered in the upper part and three- to four-layered in the lower part (Fig. 4E, F). In *Lopezia,* the mature ovule is hemianatropous, and therefore the o.i. is well developed on the funicular side also (Fig. 4D).

Thus, since subdermal initials and their derivatives contribute to the formation of the integument, the o.i. of *Lopezia* is of subdermal origin, a term used to express a mode contrasting with that in *Ludwigia.*

TRIBE FUCHSIEAE

Fuchsia jimenezii, F. microphylla subsp. *quercetorum,* and *F. radicans*—The i.i. is of dermal origin and two-layered in all species examined.

The o.i. is of subdermal origin in all species examined. Derivatives of subdermal initials participate in the formation of a major part of the o.i., and reach its apex as in *Lopezia* (Fig. 5). The thickness of the o.i. differs from species to species: three- to four-layered in *F. jimenezii,* and three- to six-layered in *F. radicans* (Fig. 5) and *F. microphylla.*

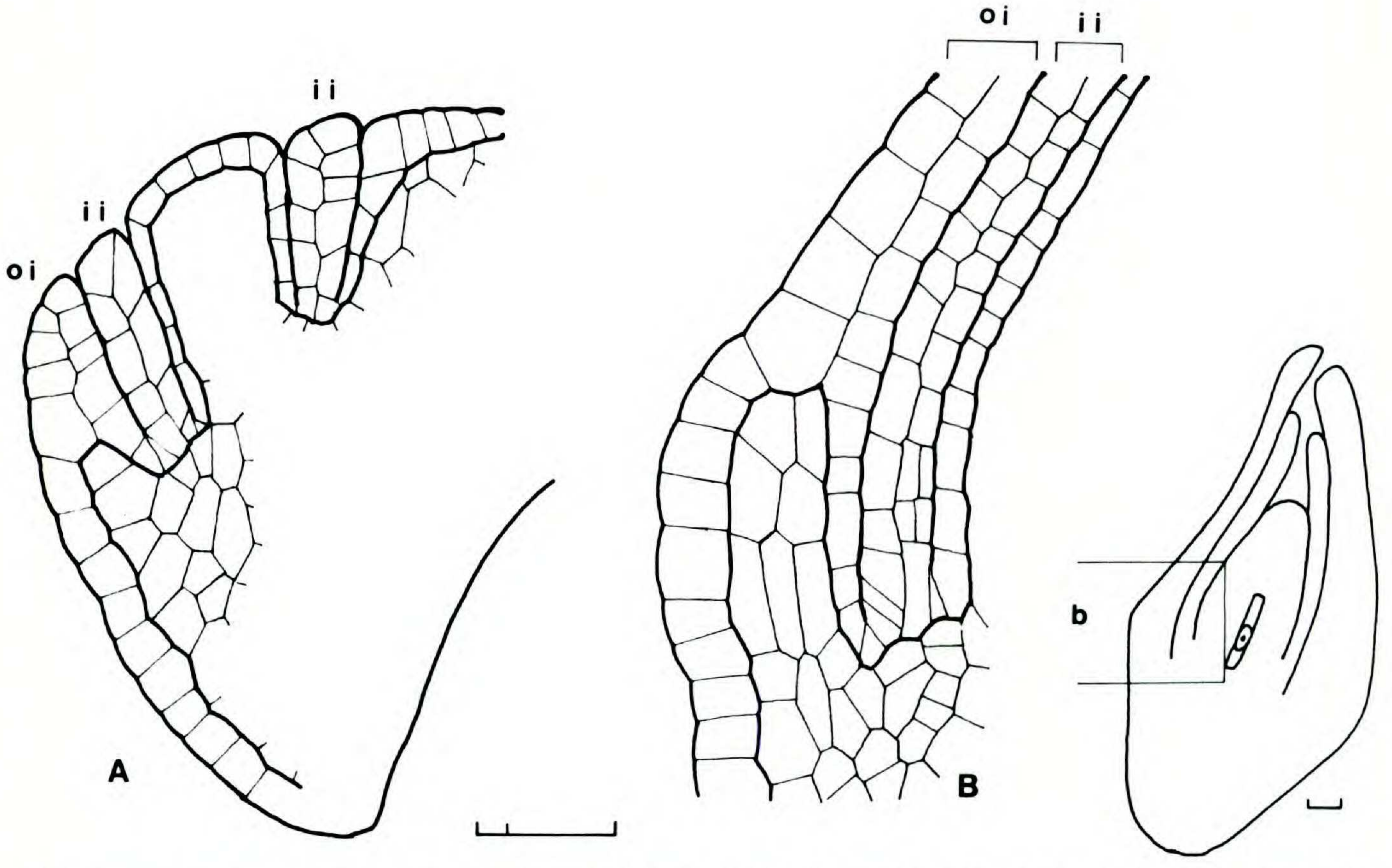


FIGURE 6. *Hauya elegans* subsp. *elegans.*—A. Median longisection of an ovule primordium.—B. Longisection of a developed ovule. Note that the o.i. is of both dermal and subdermal origin, and the derivatives of the dermal initials divide more actively than those of the subdermal ones in the formation of the o.i. Bracket scales = 100 μm.

TRIBE CIRCAEEAE

Circaea alpina subsp. *pacifica* and *C. cordata*—The i.i. is of dermal origin and two-layered in both species.

The o.i. is of subdermal origin and three- to five-layered in both species. Derivatives of subdermal initials reach the tip of the o.i. as in *Lopezia* and *Fuchsia* (Fig. 9A).

TRIBE HAUYEAE

Hauya elegans subsp. *elegans* and *H. heydeana*—The i.i. is of dermal origin and two-layered in both species (Fig. 6A, B).

The o.i. is of both dermal and subdermal origin in both species. On the antiraphial and lateral surfaces, the initiation of the o.i. commences with periclinal divisions of dermal as well as of subdermal initials (Fig. 6A). Subsequently, however, derivatives of the dermal initials divide more actively than those of the subdermal initials, so that the derivatives of the subdermal initials remain in the basal part of the o.i. on the antiraphial and lateral sides (Fig. 6B). Consequently, the mature o.i. is mostly two-layered, but three- to five-layered in its basal portion in both species (Fig. 6B).

TRIBE ONAGREAE

Gongylocarpus fruticulosus and *G. rubricaulis*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species.

Gayophytum heterozygum and *G. humile*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species.

Xylonagra arborea—Both the i.i. and the o.i. are of dermal origin and two-layered.

Camissonia californica and *C. ovata*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species.

Calylophus lavandulifolius and *C. serrulatus*—The i.i. is of dermal origin and two-layered in both species. The mode of the initiation and the subsequent growth of the o.i. is similar to that in *Hauya.* In both species the o.i. is initiated by periclinal divisions of dermal as well as of subdermal initials (Fig. 7A). Derivatives of the dermal initials divide more often than those of the subdermal initials subsequently (Fig. 7B). Ultimately, the derivatives of subdermal initials par-

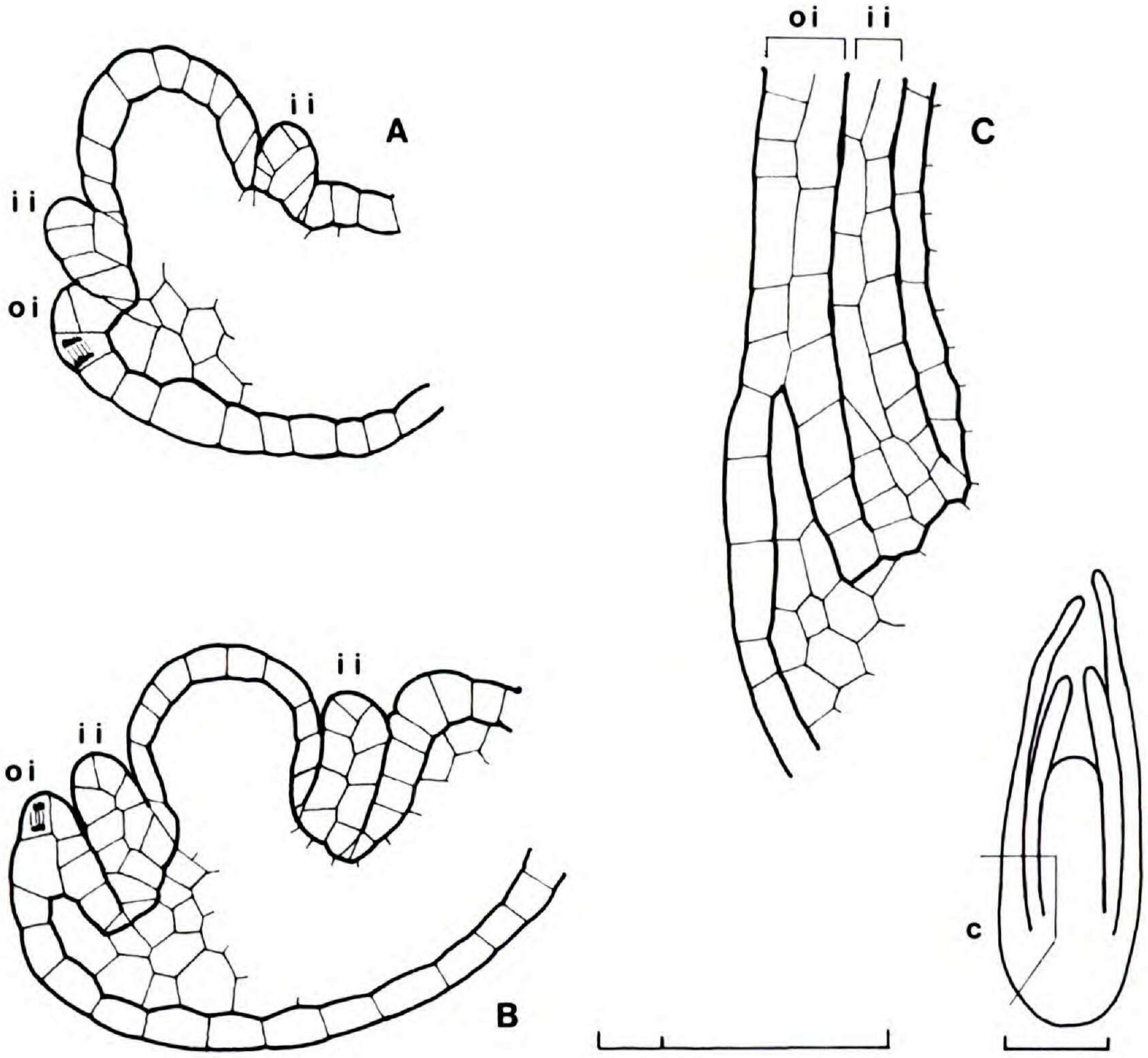


FIGURE 7. *Calylophus lavandulifolius.*—A, B. Median longisections of ovule primordia in progressively successive stages of development, showing that the o.i. is of both dermal and subdermal origin.—C. Longisection of a developed ovule. Note that the derivatives of the dermal initials divide more often than those of the subdermal ones in the formation of the o.i. Bracket scales = 100 μm.

ticipate only in the formation of the basal part of the o.i. (Fig. 7C). The mature o.i. is mostly two-layered but three- to four-layered in its basal portion (Fig. 7C).

Gaura coccinea and *G. longiflora*—The i.i. is of dermal origin and two-layered, and the o.i. of both dermal and subdermal origin in both species. The degree of participation of derivatives of subdermal initials in the formation of the o.i. closely resembles that seen in *Hauya* and *Calylophus.* Therefore, the mature o.i. is mostly two-layered but three- to four-layered in its basal portion, which is made up of derivatives of dermal and subdermal initials.

Oenothera flava subsp. *flava* and *O. villosa* subsp. *villosa*—The i.i. is of dermal origin, as Geerts (1908) observed, and two-layered in both species. On the antiraphial and lateral sides, the o.i. is of both dermal and subdermal origin in both species. Geerts (1908) mentioned that in *O. glazioviana* (= "*O. lamarckiana*"), the o.i. is produced by divisions of subdermal cells. But his figure (Geerts, 1908, table 19, fig. 3) illustrating a median longisection of the very young ovule primordium shows the o.i. swelling by divisions of derivatives of both dermal and subdermal initials. In both species examined in this paper, the dermal and the subdermal initials appear to divide almost simultaneously (Fig. 8A). In any event, at least it is obvious that the derivatives of both the dermal and subdermal initials contribute to the formation of the o.i. Of more importance to the final outcome is the fact that the derivatives of the dermal initials divide less actively than those of the subdermal ones, a feature in which *Oenothera* differs from *Hauya, Calylophus,* and *Gaura* (Fig. 8B). The mature o.i. is two-layered in the upper portion and three-layered in the lower portion (Fig. 8C).

Stenosiphon linifolius—The i.i. is of dermal origin and two-layered. The mode of initiation and the subsequent growth of the o.i. is almost identical to that in *Oenothera.* On the antiraphial and lateral sides, the o.i. is of both dermal and

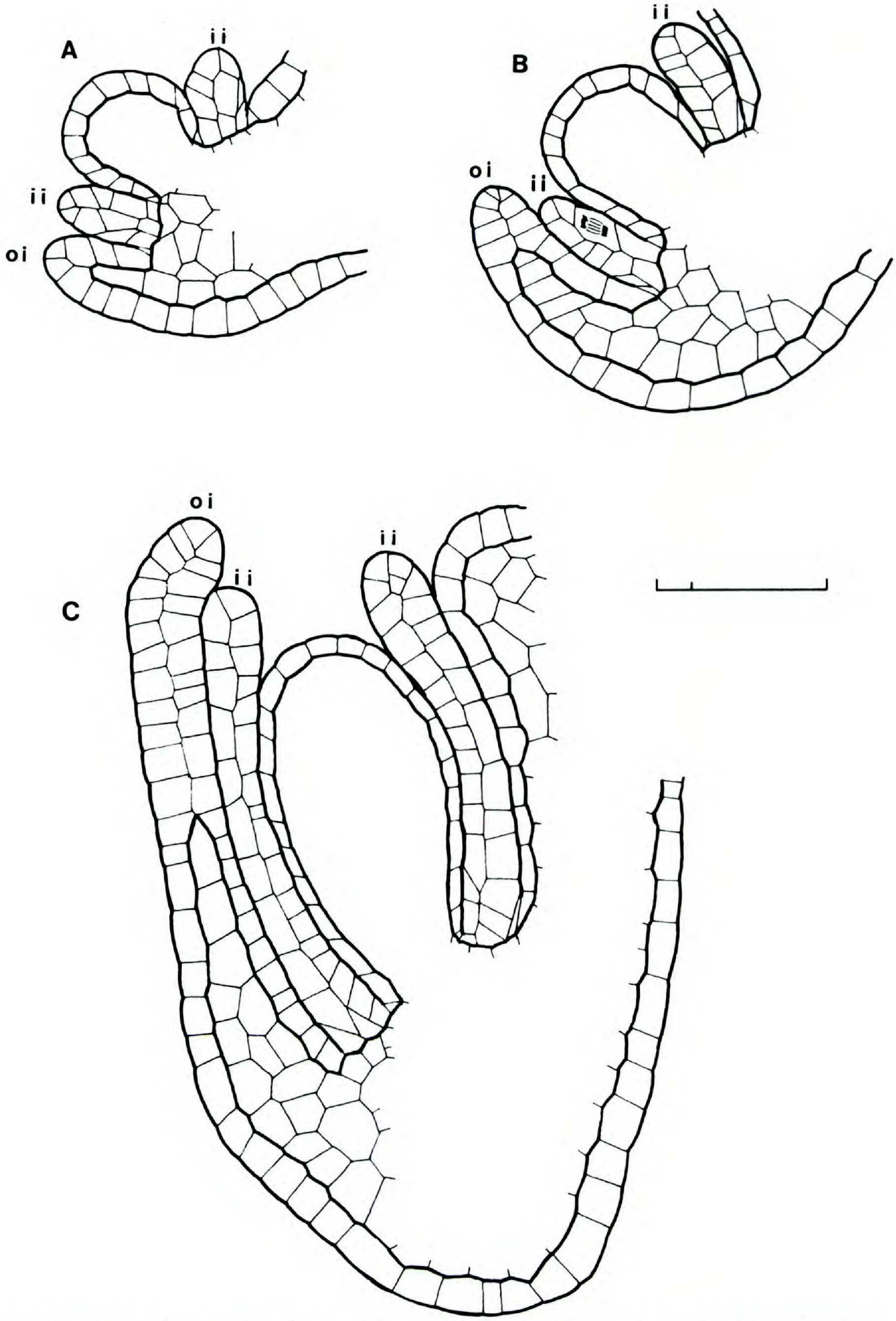
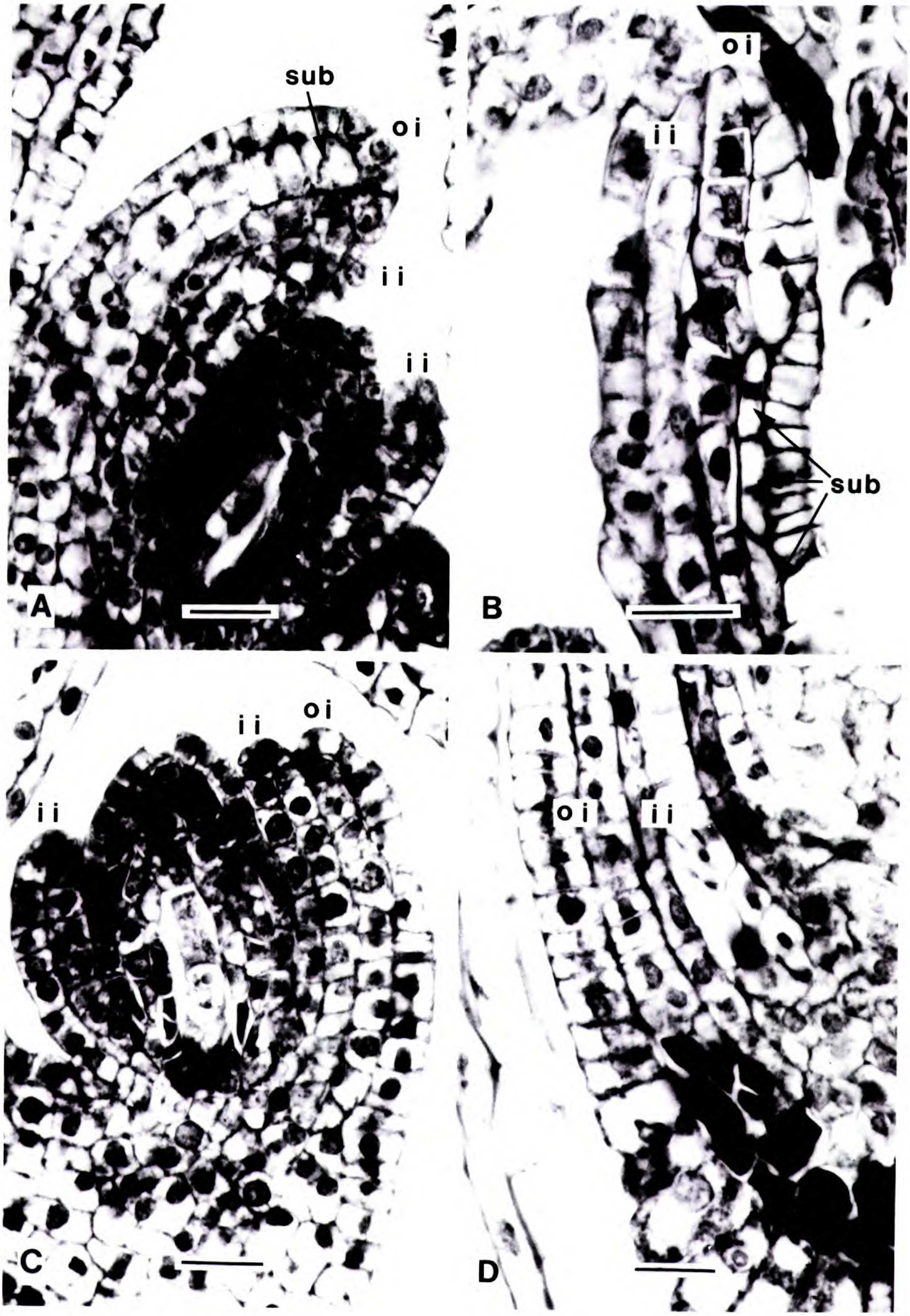


FIGURE 8. *Oenothera flava* subsp. *flava.*—A–C. Median longisections of ovule primordia in progressively successive stages of development, showing that the o.i. is of both dermal and subdermal origin. Note that the derivatives of the dermal initials divide less actively than those of the subdermal ones in the formation of the o.i. Bracket scale = 100 μm.

subdermal origin with the derivatives of the dermal initials dividing less often than those of the subdermal initials subsequently. In some ovules, the derivatives of the subdermal initials extend close to the tip of the o.i. (Fig. 9B).

Clarkia delicata and *C. tenella*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species (Fig. 9C).

Heterogaura heterandra—Both the i.i. and the o.i. are of dermal origin and two-layered.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Boisduvalia glabella and *B. subulata*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species.

Epilobium canum and *E. ciliatum*—Both the i.i. and the o.i. are of dermal origin and two-layered in both species (Fig. 9D).

DISCUSSION

In Table 1 the results of this study on the histogenesis of integuments are presented comparatively. Although all Onagraceae have a two-layered i.i. of dermal origin, four different types of o.i. are recognized with respect to mode of initiation and subsequent growth. These are found in, respectively, 1) *Lopezia* (Lopezieae), *Fuchsia* (Fuchsieae), and *Circaea* (Circaeeae), in which the o.i. is of subdermal origin, and the derivatives of the subdermal initials reach the tip of the o.i. (Fig. 10A); 2) *Oenothera* and *Stenosiphon* (Onagreae), in which the o.i. is of both dermal and subdermal origin, and the derivatives of the subdermal initials extend beyond the middle of the o.i. (Fig. 10B); 3) *Hauya* (Hauyeae) and *Calylophus* and *Gaura* (Onagreae), in which the o.i. is both dermal and subdermal origin, and the derivatives of the subdermal initials are confined to the basal part of the o.i. (Fig. 10C); and 4) *Ludwigia* (Jussiaeeae), *Boisduvalia* and *Epilobium* (Epilobieae), and *Camissonia, Gayophytum, Gongylocarpus,* and *Xylonagra* (Onagreae), in which the o.i. is of dermal origin and mostly two-layered (Fig. 10D).

In angiosperms, the i.i. is almost always of fully dermal derivation and mostly two or three cells thick (Bouman, 1974, 1984); Euphorbiaceae is the only family in which a subdermally initiated i.i. is reported (Bor & Bouman, 1974). Therefore, we could conclude that most Onagraceae have a generalized i.i. with respect to histogenesis and thickness. The amplification in thickness of the i.i. because of periclinal cell divisions, which often is characteristic of *Ludwigia,* would in that case be interpreted as a secondary characteristic (Bouman, pers. comm.).

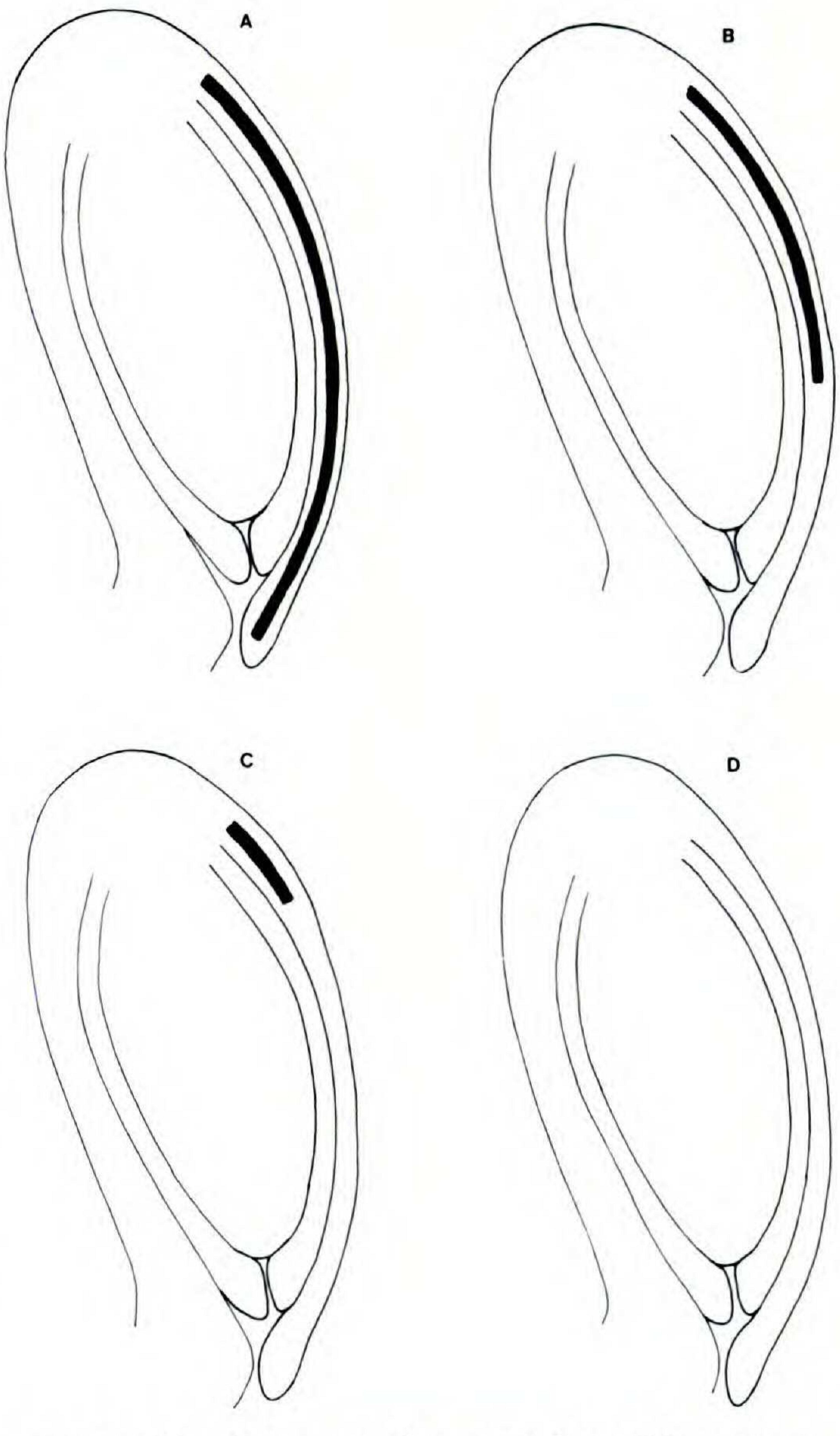

FIGURE 10. Diagrams illustrating four different types of the o.i. with respect to the developmental mode. —A. The o.i. is of subdermal origin.—B. The o.i. is of both dermal and subdermal origin with the derivatives of the subdermal initials dividing more actively than those of the dermal ones.—C. The o.i. of both dermal and subdermal origin with the derivatives of the dermal initials dividing more often than those of the subdermal ones.—D. The o.i. is of dermal origin.

Bouman (1974, 1984) has postulated that the most primitive type of angiospermous ovule has

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FIGURE 9. A. *Circaea cordata.* Median longisection of a growing ovule, showing that the o.i. is of subdermal origin and the derivatives of the subdermal initials reach up to the tip of the o.i.—B. *Stenosiphon linifolius.* Longisection of apical part of integuments. Note that, since the o.i. is of both dermal and subdermal origin, a certain part of the o.i. is made up of derivatives of both the dermal and subdermal initials. In this ovule, the derivatives of the subdermal initials extend close to the tip of the o.i.—C. *Clarkia tenella.* Median longisection of a growing ovule showing two-layered i.i. and o.i. of dermal origin.—D. *Epilobium ciliatum.* Longisection of a basal part of a developed ovule showing two-layered i.i. and o.i. of dermal origin. sub, derivatives of the subdermal initials. Scales = 20 μm.

a subdermally initiated o.i. and that thick o.i.'s are more primitive than thinner ones. In light of Bouman's principles, the histogenesis of the o.i.'s of *Lopezia, Fuchsia,* and *Circaea* would be considered the most primitive found in Onagraceae, because in these genera the o.i. is entirely of subdermal origin. At least some species of all three genera have the original basic chromosome number for the family, $n = 11$, and generalized chromosome structure (Kurabayashi et al., 1962; Raven, 1979). As indicated by Eyde and Morgan (1973), *Lopezia, Fuchsia,* and *Circaea* have no evident direct relationship to one another. For example, although both have reduced numbers of flower parts, *Lopezia* differs from *Circaea* in its anther disposition and nectary morphology. *Fuchsia,* despite its mostly red, bird-pollinated flowers and unique fleshy fruits, may be the modern genus closest to the ancestors of most Onagraceae. The pattern of histogenesis of its integuments is in accordance with such a conclusion.

The tribe Onagreae, with ten genera, exhibits three patterns with respect to the histogenetic characters of integuments (see Table 1). Based on Bouman's (1974, 1984) postulates, *Oenothera* and *Stenosiphon* are more primitive than other members of the tribe in that their o.i. retains a stronger tendency to entirely subdermal initiation (see Fig. 10B). *Calylophus* and *Gaura* appear to be more specialized than *Oenothera* and *Stenosiphon,* and less specialized than other Onagreae, in that their o.i. still retains a weak tendency to an entirely subdermal initiation (see Fig. 10C). The remaining six genera, *Camissonia, Gayophytum, Gongylocarpus, Xylonagra, Clarkia,* and *Heterogaura,* in which the o.i. has no tendency to a subdermal initiation (see Fig. 10D), constitute a third group, which on the basis of this feature would be considered the most advanced in the tribe.

It is almost certain that the patterns of integumental histogenesis found in these three groups were derived from one in which the o.i. was entirely of subdermal origin, like that which persists in *Fuchsia, Lopezia,* and *Circaea.* It is not, however, possible to determine whether they were derived sequentially or independently on the basis of this characteristic alone. *Gongylocarpus* is the only genus of Onagreae that retains the original basic chromosome number for the family, $n = 11$, but it has a highly specialized pattern of ovary development (Carlquist & Raven, 1966). If it is an offshoot from the line leading to *Camissonia, Gayophytum,* and *Xylonagra* (Raven, 1964, 1969), then, since all of these genera have identical specialized patterns of integumentary histogenesis in agreement with their distinctive stigma morphology, it would be implied that there is no direct relationship between this line and other Onagreae. Certainly, these relationships make it appear that the reduced basic chromosome number, $n = 7$, must have been derived separately in this line and in the other parts of the tribe.

The large genus *Oenothera* (about 125 species) is identical to the monotypic *Stenosiphon* in the pattern of histogenesis of its integuments, as it is in stigma morphology and other features. Thus the close relationship between these genera first postulated by Raven (1964) is confirmed, but the relationship between these genera and others within the tribe remains obscure. The basic chromosome number, $n = 7$, in these genera certainly seems to have evolved independently from the same number in other genera of Onagreae.

Clarkia and *Heterogaura* share the specialized feature of the o.i. with the first group discussed above. If they are directly related to *Calylophus* and *Gaura,* as claimed by Raven (1964, 1969) on the basis of the thick partitions that divide the sporogenous tissue of their anthers into packets, it would have to be assumed that the pattern of development of their o.i.'s was independently derived from that in the "*Gongylocarpus*—group"; if not, then their specialized anthers and stigmas must have evolved within that group.

Hauya ($x = 10$), now placed in a separate tribe Hauyeae (Raven, 1979) on the basis of the many features, all apparently retained primitive ones, in which it differs from all genera currently assigned to Onagreae, was formerly grouped with *Calylophus, Clarkia, Gaura,* and *Heterogaura* on the basis of its divided sporogenous tissue. The histogenesis of its integuments is identical to that in *Calylophus* and *Gaura,* differing sharply from the more advanced condition found in *Clarkia* and *Heterogaura* and the more primitive one in *Oenothera* and *Stenosiphon.* This may indicate that *Calylophus* and *Gaura* (both $x = 7$) were derived from a phylogenetic line separate from that leading to other Onagreae, and that *Hauya* may be a surviving early evolutionary offshoot of that line. The relationships of the different genera of Onagreae and their affinity to *Hauya* will be discussed in more detail in a separate paper focusing on the exact nature of the divided sporogenous tissue in the Onagraceae (Tobe & Raven, 1985).

The remaining two tribes of the family, Jussiaeeae (*Ludwigia* only) and Epilobieae (*Epilobium* and *Boisduvalia*), share with six genera of Onagreae the most advanced type of integumentary histogenesis in the family, with a two-layered o.i. of dermal origin. Fundamental floral differences discussed by Eyde (1977, 1978, 1981) make it clear that *Ludwigia* represents a phylogenetic line different from all other Onagraceae, so that it must have evolved its specialized o.i. independently. There appears to be no evidence to suggest a close grouping of Epilobieae with any genera of Onagreae, so that we may assume that a two-layered o.i. of dermal origin evolved independently in it, in the *Gongylocarpus*-line, and perhaps also in *Clarkia–Heterogaura*—thus three or even four times within Onagraceae. A similar condition has evolved independently in various angiosperms, as for example, Lactoridaceae among "Polycarpicae" (Bouman, 1971).

Within the family, *Ludwigia peploides* (sect. *Oligospermum*) is distinct from all other species in having a three-layered o.i. instead of a two-layered one. Eyde (1977), pointing out that sect. *Oligospermum* stands apart from other ludwigias because of its characteristic aerenchymatous tissue surrounding the endocarp, inferred that this section seems to have diverged early from the other ludwigias and developed its own aquatic adaptations. The characteristic developmental mode of its integuments, unique in Onagraceae, constitutes additional evidence supporting his hypothesis.

This comparative study covering the whole family enables us to evaluate the histogenetic characters of integuments. As has been described and seen in Table 1, this character is nearly genus-specific. Even in cases in which the seed coat or testa histology differs from species to species (e.g., in *Gongylocarpus*, Carlquist & Raven, 1966), the mode of initiation and the subsequent growth of the integuments is consistent within a genus. In view of this, detailed comparisons of this character have made it possible to evaluate the affinities of genera and to consider their relative states of advancement.

LITERATURE CITED

BOER, R. DE & F. BOUMAN. 1972. Integumentary studies in the Polycarpicae. II. *Magnolia stellata* and *Magnolia virginiana*. Acta Bot. Neerl. 21: 617–629.

——— & ———. 1974. Integumentary studies in the Polycarpicae. III. *Drimys winteri* (Winteraceae). Acta Bot. Neerl. 23: 19–27.

BOESEWINKEL, F. D. 1977. Development of ovule and testa in Rutaceae. I. *Ruta, Zanthoxylum* and *Skimmia*. Acta Bot. Neerl. 26: 193–211.

———. 1978. Development of ovule and testa in Rutaceae. II. Some representatives of the Aurantioideae. Acta Bot. Neerl. 27: 342–354.

——— & F. BOUMAN. 1967. Integument initiation in *Juglans* and *Pterocarya*. Acta Bot. Neerl. 16: 86–101.

——— & ———. 1978. Development of ovule and testa in Rutaceae. II. The unitegmic and pachychalazal seed of *Glycosmis* cf. *arborea* (Roxb.) D.C. Acta Bot. Neerl. 27: 69–78.

BOR, J. & F. BOUMAN. 1974. Development of ovule and integuments in *Euphorbia milii* and *Codiaeum variegatum*. Phytomorphology 24: 280–296.

BOUMAN, F. 1971. Integumentary studies in the Polycarpicae. I. Lactoridaceae. Acta Bot. Neerl. 20: 565–569.

———. 1974. Developmental studies of the ovule, integuments and seed in some angiosperms. Thesis. Univ. of Amsterdam.

———. 1977. Integumentary studies in the Polycarpicae. IV. *Liriodendron tulipifera* L. Acta Bot. Neerl. 26: 213–223.

———. 1978. Integumentary studies in the Polycarpicae. V. *Nigella damascena* L. Acta Bot. Neerl. 27: 175–182.

———. 1984. The ovule. Pp. 123–157 *in* B. M. Johri (editor), Embryology of Angiosperms. Springer-Verlag, Heidelberg.

CARLQUIST, S. 1975. Wood anatomy of Onagraceae, with notes on alternative modes of photosynthate movement in dicotyledon woods. Ann. Missouri Bot. Gard. 62: 386–424.

———. 1977. Wood anatomy of Onagraceae: additional species and concepts. Ann. Missouri Bot. Gard. 64: 627–637.

———. 1982 [1983]. Wood anatomy of Onagraceae: further species; root anatomy; significance of vestured pits and allied structures in dicotyledons. Ann. Missouri Bot. Gard. 69: 755–769.

——— & P. H. Raven. 1966. The systematics and anatomy of *Gongylocarpus* (Onagraceae). Amer. J. Bot. 53: 378–390.

EYDE, R. H. 1977 [1978]. Reproductive structures and evolution in *Ludwigia* (Onagraceae). I. Androecium, placentation, merism. Ann. Missouri Bot. Gard. 64: 644–655.

———. 1978 [1979]. Reproductive structures and evolution in *Ludwigia* (Onagraceae). II. Fruit and seed. Ann. Missouri Bot. Gard. 65: 656–675.

———. 1981. Reproductive structures and evolution in *Ludwigia* (Onagraceae). III. Vasculature, nectaries, conclusions. Ann. Missouri Bot. Gard. 68: 470–503.

———. 1982 [1983]. Evolution and systematics of the Onagraceae: floral anatomy. Ann. Missouri Bot. Gard. 69: 735–747.

——— & J. T. MORGAN. 1973. Floral structure and evolution in Lopezieae (Onagraceae). Amer. J. Bot. 60: 771–787.

GEERTS, J. M. 1908. Beitrage zur Kenntnis der cytologischen Entwicklung von *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Ges. 26: 608–614.

JOHANSEN, D. A. 1934. Studies on the morphology of the Onagraceae. VIII. *Circaea pacifica*. Amer. J. Bot. 21: 508–510.

KEATING, R. C. 1982 [1983]. The evolution and systematics of Onagraceae: leaf anatomy. Ann. Missouri Bot. Gard. 69: 770–803.

KURABAYASHI, M., H. LEWIS & P. H. RAVEN. 1962. A comparative study of mitosis in the Onagraceae. Amer. J. Bot. 49: 1003–1026.

O'NEAL, C. E. 1923. A study of the embryo sac development and accompanying phenomena in *Oenothera rubrinervis*. Bull. Torrey Bot. Club 50: 133–146.

PLITMANN, U., P. H. RAVEN & D. E. BREEDLOVE. 1973. The systematics of Lopezieae (Onagraceae). Ann. Missouri Bot. Gard. 62: 621–646.

RAVEN, P. H. 1963. The Old World species of *Ludwigia* (including *Jussiaea*), with a synopsis of the genus (Onagraceae). Reinwardtia 6: 327–427.

———. 1964. The generic subdivision of Onagraceae, tribe Onagreae. Brittonia 16: 276–288.

———. 1969. A revision of the genus *Camissonia* (Onagraceae). Contr. U.S. Natl. Herb. 37: 161–396.

———. 1976. Generic and sectional delimitation in Onagraceae, tribe Epilobieae. Ann. Missouri Bot. Gard. 63: 326–340.

———. 1979. A survey of reproductive biology in Onagraceae. New Zealand J. Bot. 17: 575–593.

——— & D. P. GREGORY. 1972. Observations of meiotic chromosome in *Gaura* (Onagraceae). Brittonia 24: 71–86.

——— & W. TAI. 1979 [1980]. Observations of chromosomes in *Ludwigia* (Onagraceae). Ann. Missouri Bot. Gard. 66: 862–879.

ROTH, I. 1957. Die Histogenese der Integumente von *Capsella bursa-pastoris* und ihre morphologische Bedeutung. Flora 145: 212–235.

SKVARLA, J. J., P. H. RAVEN & J. PRAGLOWSKI. 1975. The evolution of pollen tetrads in Onagraceae. Amer. J. Bot. 62: 6–35.

———, ———, W. F. CHISSOE & M. SHARP. 1978. An ultrastructural study of viscin threads in Onagraceae pollen. Pollen & Spores 20: 5–143.

TÄCKHOLM, G. 1914. Zur Kenntnis der Embryosackentwicklung von *Lopezia coronata* Andr. Svensk Bot. Tidskr. 8: 223–234.

TOBE, H. & P. H. RAVEN. 1985. The evolution of polysporangiate anthers in Onagraceae. Amer. J. Bot. (in press).

WARMING, M. E. 1878. De l'ovule. Ann. Sci. Nat. Bot., Ser. 6, 5: 177–266.